



Faculty of Resource Science and Technology

**EFFECT OF DIFFERENT CONCENTRATIONS OF
COLLETOTRICHUM GLOEOSPORIOIDES ON DISEASE
DEVELOPMENT AND DISEASE SEVERITY ON LEAVES OF
RUBBER TREE (*HEVEA BRASILIENSIS*)**

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**Bachelor of Science with Honours
(Plant Resource Science and Management)
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APPROVAL SHEET

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“I declare that I have read this work and in my opinion, this work is adequate in terms of scope and quality of the purposes of awarding a Bachelor’s degree of Science with Honours (Plant Resource Science and Management)”.



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DECLARATION

I hereby declare that this Final Year Project report entitled 'Effect of Different Concentrations of *Colletotrichum gloeosporioides* on Disease Development and Disease Severity on Leaves of Rubber Tree (*Hevea brasiliensis*)' is based on my original work except for the citations and quotations which have been duly acknowledged. I also declare that no portion of this research work has been submitted to support the application of other degree or qualification at any other universities or institutions of higher learning.



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LIST OF ABBREVIATIONS

Carnation Leaf Agar	CLA
<i>Colletotrichum</i> leaf disease	CLD
Critical Point Drying	CPD
Czapek-Dox Agar	CDA
Maize Agar	MMA
Maize Dextrose Yeast Extract Agar	MDYEA
Maize Soybean Dextrose Agar	MSDA
One-way Analysis of Variance	ANOVA
PDA Plus Yeast Extract	PDAY
Potato Carrot Agar	PCA
Potato Dextrose Agar	PDA
Tap Water Agar	TPA
Water Agar	WA
Water Hyacinth Dextrose Agar	WHDA

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ABSTRACT

Disease development on leaves of rubber seedlings when inoculated with different inoculum concentrations of *Colletotrichum gloeosporioides* were observed. The leaves used were manipulated in 16 ways in a combination of wounding and sterilizing the attached and detached leaves. Inoculum concentrations of 10^2 , 10^4 and 10^6 spores/ml were inoculated on these leaves. Disease development was recorded based on number of spot observed on the leaf surface for four weeks. Inoculum concentration of 10^6 spores/ml resulted in the most significant disease development (number of spots). The inoculum concentration which recorded most number of spots on the leaf surface from the effect of concentration experiment was inoculated on leaves and tested on the severity of disease when left in different durations of continuous moist condition. Number of spots on leaf surface was highest at fourth week after inoculation. Fungal response was observed for 24, 48, 72, 96 and 120 hours of wetness duration after inoculation. It was recorded to have required a minimum of five days for penetration and colonization of host.

Keywords: attached, detached, sterilizing, wounding

ABSTRAK

Perkembangan penyakit di atas daun-daun anak pokok getah apabila diinokulasikan dengan kadar konsentrasi inokula *Colletotrichum gloeosporioides* yang berlainan telah dipantau. Faktor ini telah dikaji ke atas daun-daun anak pokok getah yang telah dimanipulasikan dalam 16 cara iaitu dari segi mencederakan dan mensterilkan daun yang telah dipetik dari pokoknya dan yang masih dikekalkan pada pokok. Kadar konsentrasi inokula 10^2 , 10^4 and 10^6 spora/ml telah diinokulasikan ke atas daun-daun tersebut. Perkembangan penyakit dicatat berdasarkan bilangan titik hitam yang ditemui di atas permukaan daun dalam jangka masa empat minggu. Didapati bahawa kadar konsentrasi inokula 10^6 spora/ml telah mengakibatkan perkembangan penyakit paling ketara. Kadar konsentrasi inokula yang telah mengakibatkan paling banyak bilangan titik hitam yang ditemui di atas permukaan daun daripada eksperimen kesan kadar konsentrasi inokula telah digunakan untuk menginokulasi daun-daun dalam eksperimen untuk mengesan kadar keterukan penyakit di permukaan daun apabila dibiarkan dalam keadaan basah selama empat minggu. Kajian ini menunjukkan bahawa paling banyak titik hitam direkod pada minggu keempat setelah diinokulasi. Tindak balas kulat telah dipantau selama 24, 48, 72, 96 and 120 jam jangka masa basah. Ia didapati bahawa sekurang-kurangnya 120 jam jangka masa basah diperlukan bagi proses penembusan dan kolonisasi daun tersebut.

Kata kunci: dikekalkan, dipetik, mensterilkan, mencederakan

INTRODUCTION

1.1 General Introduction

Hevea brasiliensis Müell.Arg. or the rubber tree is also known as the Para rubber tree and common rubber tree. This species belongs to the family Euphorbiaceae (Lusweti et al., 2011). The most essential feature of this tree is the white latex formed when the bark is cut or tapped. It is this white latex which is vulcanized to form rubber (Navie and Adkins, 2008). The rubber tree is prone to a wide range of foliar diseases, which causes yield losses of rubber latex. *Colletotrichum* leaf disease (CLD) is considered as one of the major foliar diseases that causes declining yields of rubber in Asia (Thambugala and Deshappriya, 2009).

According to Bailey and Jeger (1992), *Colletotrichum* is one of the most common and important genus of plant pathogenic fungi. At present, there have been about 900 species described or assigned to *Colletotrichum*. Based on perceived scientific and economic importance, this genus was recently voted the eighth most important group of plant pathogenic fungi in the world (Cannon et al., 2012). Almost all the crops grown throughout the world is susceptible to one or more species of *Colletotrichum*. This genus causes economically significant diseases of cereals and grasses, legumes, vegetables and perennial crops, such as tree fruits and rubber.

Colletotrichum gloeosporioides usually infects any green parts of the rubber tree such as the young leaves, green shoots, and pods (Jayasinghe et al., 1996). Anthracnose and leaf blight of rubber is one of the most serious diseases caused by *C. gloeosporioides* that affects the flush of new leaves produced following the 'wintering' effect. This occurs when the rubber tree, which is deciduous, loses its leaves during the dry season. The new leaves produced are

vulnerable to infection and an abundant of vulnerable host tissues combined with wet conditions favours epidemic development of the disease (Waller, 1992).

Besides, according to Bailey et al. (1992), the *Colletotrichum* sp. is a very dangerous pathogen because of its variable mode of infection. Several modes of penetration are possible, which are through natural openings such as stomata, through wounds and by direct penetration of the cuticular barrier. The most common method of penetration is by direct penetration of plant cuticles. Even though infection through wound is uncommon, for some diseases such as crown and finger stalk rot of banana, it is essential (Wang, 2009).

The infection strategies of plant pathogenic fungi involve several stages such as attachment and germination of propagules, differentiation into specialized pre-penetration structures such as appressoria, penetration of host cells, development of infection hyphae and colonization of plant tissues (Morin et al., 1996; Gomes et al., 2012). There are a few factors that encourage growth and infection process of this fungus such as inoculum concentration, wetness duration, temperature, age of plant, resistant plant cultivar, pH, nutrient and light (University of Sydney, 2004).

Due to the variable ways this fungus can penetrate plants, it is vital to study the response of *C. gloeosporioides* under different parameters to be able to perform the correct method of control and at the correct time (Dean and Kuc, 1986; Bailey and Jeger, 1992). Therefore, this research is focused on the disease development on leaves of rubber tree when inoculated with different concentrations of *C. gloeosporioides* inoculum and when left in different durations of continuous moist condition.

1.2 Problem Statement

C. gloeosporioides causes diseases such as *Colletotrichum* leaf disease and anthracnose on rubber tree. *C. gloeosporioides* inoculum concentration and wetness duration are among the factors that facilitate disease development and fungal response on rubber tree (Thambugala and Deshappriya, 2009). Due to this, more information is needed on the disease development on the leaves of the rubber seedlings in Malaysia when inoculated with different concentrations of inoculum and disease severity when the inoculated leaves are left in different durations of continuous moist condition. To date, many researches have been done on crops such as fruits and vegetables but fewer studies have been conducted about the response of this fungus on rubber tree. Research on disease development on attached and detached leaves are also limited. There is difference in results for experiment done in lab and field (Liu et al., 2007). Furthermore, few papers have been published on the response of *C. gloeosporioides* on wounded and unwounded leaves. Disease development on wounded crops showed higher severity. Therefore, it is suspected that wounding also affects disease severity on rubber seedling leaves. Besides, less study have been done on effect of sterilized and unsterilized leaves on disease development on rubber seedlings.

Detailed knowledge of the infection process of *C. gloeosporioides* may provide helpful information to direct research towards optimizing the suitable fungicide to eradicate this species. Currently, anthracnose in Malaysia is mainly controlled by scheduled application of fungicides on seedlings in plantation area because of lack of knowledge of the factors affecting the disease severity under Malaysia's conditions. Thus, the significance of this study will be to allow rubber tree planters to forecast a system that could regulate timing and amount of fungicide sprays on the seedlings and allow the application of more effective management

strategies for the diseases caused by this fungus on the rubber trees in Malaysia. Therefore, the results of these investigations will enable the control of the disease to be improved in the rubber estate of Malaysia (Guyot et al., 2004).

1.3 Objectives

The objectives of this research are:

1. To study the disease development on the leaves of rubber tree when different concentrations of *C. gloeosporioides* are inoculated.
2. To analyse the disease severity on the leaves of rubber tree when *C. gloeosporioides* is inoculated and left in different durations of continuous moist condition.
3. To research the fungal response of *C. gloeosporioides* when inoculated on the leaves of rubber tree and left for different wetness durations.
4. To investigate the effect of wounding and sterilising detached and attached leaves of rubber tree on disease development and fungal response.

LITERATURE REVIEW

2.1 Diseases Caused by *Colletotrichum* sp. on Crops

Colletotrichum sp. is known to cause many diseases. Post-harvest rots, anthracnose spots and blights of aerial plant parts are among the diseases caused by this fungus (Freeman et al., 1998). Disease symptoms of anthracnose typically include sub-circular or angular depressed black lesion, and in which erumpent salmon-pink spore masses develop (Bailey and Jeger, 1992; Nelson, 2008). Besides, symptoms first appear as sunken, water-soaked lesions that expand rapidly on fruit. Generally, the fully expanded lesions are soft, sunken and range in colour from dark red to tan to black (Hong and Hwang, 1998). On the other hand, foliage and stem symptoms appear as small, irregularly shaped grey-brown spots with dark brown edges. Flowers are also infected, which is called blossom blight. The blight can destroy flowers and young fruits and cause complete crop failure (Phoulivong, 2011).

Losses are caused by the effects of disease at different growth stages of the plant. Plants are affected during establishment and vegetative growth, through loss of inflorescences and premature fruit fall, through photosynthetic and other physiological dysfunctions, and through post-harvest losses. These losses are caused by the phenomenon of latent or quiescent infection (Bailey and Jeger, 1992; Hong and Hwang, 1998). As a result, major economic losses, especially of fruits, vegetables, and ornamentals plants occur (Kekuda et al., 2014). *Colletotrichum* sp. is also very damaging to important food crops, such as cassava, bananas, sorghum, and pulses, grown by subsistence farmers in developing countries throughout the tropics and subtropics (*Colletotrichum Database*, 2010). Above-ground plant parts are affected at all stages of maturity, from seedlings to mature plants and seed (Chongo and Bernier, 2000). Even though these pathogens cause damage to most parts of plants including

roots, stems, leaves, flowers, and fruits, they are often highly specific to individual tissues. In fact, many are also specific to particular species or cultivars (Bailey et al., 1992).

2.2 Methods Applied to Control the Disease

Therefore, constructive methods need to be taken to control this fungus. Effective control of *Colletotrichum* diseases generally involves the use of one or a combination of methods. The methods are using resistant cultivars, cultural, chemical, and biological control using antagonistic organisms (Wharton and Deiguez-Uribeondo, 2004).

According to Nelson (2008), resistant cultivars of mango fruits have been found in Malaysia and India. However, the varieties which are favoured for commercial production based on flavour, colour, texture, and high shelf life properties are often the most susceptible. Apart from that, according to Prior et al. (1992), cultural control is another method used for disease control. Cultural control includes sanitation method. Hygienic practices such as removal and burning of old leaves and infected stalks can help deter *Colletotrichum* sp. infection. On the other hand, based on a study by Filoda (2008), chemical control including repeated applications of fungicide is currently the only effective way to control anthracnose and these have been used successfully in most countries where the crop is grown commercially. Also, as stated by Jeffries and Koomen (1992), biological control using *Bacillus subtilis* B-3 as the antagonist have been used against *Colletotrichum gloeosporioides* on mango tree. Besides, *Bacillus subtilis* HK-CSM-1 is also used as biological control of *Colletotrichum* sp. (Ryu et al., 2014).

Therefore, the applicability of control strategies much depends on the characteristics of the crops on which they are being used and the disease at which they are targeted (Wharton and Deiguez-Uribeondo, 2004).

2.3 Infection process of *Colletotrichum* sp.

To anticipate the disease occurrence, it is vital to understand the infection process of *Colletotrichum* sp. Based on a study by Coates et al. (1993), within 48 hours of inoculation the majority of conidia present on the surface of avocado fruit had germinated to form germ tubes and appressoria. A central germ pore was visible in all appressoria in surface view examined at 48 hours after inoculation.

Other than that, Morin et al. (1996) observed on leaves of Malvaceae weed that after 3 to 4 hours of inoculation, conidia germinates by producing a single germ-tube which always emerged at a distance from the region of the former point of attachment of the conidium with the conidiophore. At 5 to 6 hours after inoculation, germ-tubes had differentiated at their tip into simple, globose to spherical appressoria which had not melanized yet. Then, after 10 to 12 hours, a septum developed between the appressorium and the conidium or germ-tube. Basically, only after 31 to 36 hours after inoculation the first signs of penetration on the hosts were observed.

On the other hand, Wang (2009) observed on leaves of lentil that after 24 hours post inoculation, infection vesicles were found inside the epidermal cells of the host. Between 36 to 60 hours post inoculation, larger primary hyphae had developed within epidermal cells.

2.4 Factors that Affect *Colletotrichum* sp. Infection and Growth

Factors that affect *Colletotrichum* sp. growth and infection include inoculum concentration, wetness duration, temperature, age of plant, level of host resistance, pH, nutrient and light.

According to Allen (1992), inoculum concentration influences infection development of the fungus. The most prominent effect is that when inoculum concentration is increased, the lag phase before infection starts is decreased. Therefore, it increases the rate of development of infection. Very low inoculum concentration may not result in a plateau of infection for a long period.

Generally, moisture is an important weather element for fungal pathogens infecting aerial plant parts such as leaves. An epidemic is likely when the temperature is conducive and the moisture requirements of a pathogen on a susceptible host are fully met for enough time. This potential role of moisture has been exploited frequently as a foundation for disease forecasting. A number of researchers have demonstrated the role of host tissue wetness in infection by pathogens for different pathogens, and the wetness duration requirements range from 0.5 to more than 100 h (Jhorar, 1998).

Temperature also affects the infection and growth of this fungus. Effect of temperature is directly related to the chemical reactions within the fungal cells. For optimal growth, temperatures must be in a range that allows the most effective progress of the chemical reactions which are needed for growth. Chemical reactions occur less efficiently as temperatures progress above the optimum temperature. This causes slow growth of the fungus. If the temperature persists to be high, it can reach a point where growth stops. Heat also

damages cell components of the fungus. Besides, enzymes undergo structural changes and denature when heated to its limit of tolerance (Burge, 2006).

According to Chongo and Bernier (2000), age of plant affects fungi infection and growth. Younger plants are more prone to fungal infection. This is due to the softer, developing tissues of the plant which is not yet resistant to fungal attacks. This is proven when younger plants which are grown together with older plants get infected by fungi earlier even though fungus was inoculated on the older plant.

Based on a study by Isaac (1992), infection and growth of this fungus is influenced by the level of host plant resistance. Plants produce phytoalexin which causes resistance of certain cultivar towards pests and diseases. After inhibition of an invading fungus, hypersensitive response in infected plants and phytoalexin synthesis occurs. This phytoalexin affects the growth of fungi by inhibiting germ tube elongation, colony growth and dry weight accumulation.

Another factor that influences growth and infection of this fungus is pH. Fungi change the pH of their surrounding as they grow. Some species increase the pH whereas others decrease pH of its medium. pH of the medium is important because it influences availability of mineral, enzyme activity and function of membrane. Generally, fungi can tolerate a wide range of pH (University of Sydney, 2004).

Nitrogen also plays a role in infection and growth of this fungus. Nutrient is required for the formation of amino acids and purines in the fungus. Many fungi utilise nitrate. However, a few fungi also require ammonium, amides, amino acids or peptides to sustain life. Total carbon and nitrogen uptake must be balanced and is critical for fungal growth and

development. Once carbon and nitrogen requirements are met, the rate of growth is determined by availability of other minerals. On the other hand, conditions of high nutrient level have no effect on the sporulation of fungi (University of Sydney, 2004).

Light is another factor that affects infection and growth of fungus. Light has limited influence on vegetative growth of individual hyphae. However, it has more effect on the reproductive structure development and sporulation of fungi. The intensity and wavelength of light are important and can stimulate or inhibit its reproduction (Isaac, 1995).

2.4.1 Inoculum Concentration

One of the main factors that facilitate *Colletotrichum* sp. infection and growth is inoculum density. Up till now, according to Diéguez-Uribeondo et al. (2011), disease severity increased exponentially with increasing concentration of inoculum. For instance, an increase in lesion number and size of necrotic lesion diameter was observed on cucumber plant as inoculum concentration was increased (Dean and Kúc, 1986). Disease incidence on immature fruit and flowers for the cultivars increased with an increase in inoculum concentration from 0 to 10^6 conidia/ml (Forcelini, 2013). This observation is also true in other pathosystem such as on tomato fruit, round-leaved mallow and velvet leaf's seeds, and Azalea leaves. (Dillard, 1989; Makowski, 1993; Bertetti et al., 2009).

However, previous studies by Thambugala and Deshappriya (2009) suggest that there is an optimum concentration of inoculum needed to observe the response of *Colletotrichum* sp. Too high or too low concentration of inoculum will not give satisfactory result. This occurs as a result of self-inhibition showed by the conidia of *Colletotrichum* sp. at high conidial